FEATURED ARTICLE

Extra-nigral Pathological Conditions Are Common in Parkinson's Disease With Freezing of Gait: An *In Vivo* Positron Emission Tomography Study

Nicolaas I. Bohnen, MD, PhD,[1,2,3]* Kirk A. Frey, MD, PhD,[1,2] Stephanie Studenski, MD, MPH,[4] Vikas Kotagal, MD,[2] Robert A. Koeppe, PhD,[1] Gregory M. Constantine, PhD,[5] Peter J.H. Scott, PhD,[1] Roger L. Albin, MD,[2,3,6] and Martijn L.T.M. Müller, PhD[1]

[1]*Department of Radiology, Division of Nuclear Medicine, University of Michigan, Ann Arbor, MI, USA*
[2]*Department of Neurology, University of Michigan, Ann Arbor, MI, USA*
[3]*Neurology Service and GRECC, VAAAHS, Ann Arbor, MI, USA*
[4]*Division of Geriatrics, Department of Internal Medicine, University of Pittsburgh, Pittsburgh, PA and Pittsburgh VA GRECC, Pittsburgh, PA, USA*
[5]*Departments of Mathematics and Statistics, University of Pittsburgh, Pittsburgh, PA, USA*
[6]*Michigan Alzheimer Disease Center, Ann Arbor, MI, USA*

ABSTRACT: Cholinergic denervation has been associated with falls and slower gait speed and β-amyloid deposition with greater severity of axial motor impairments in Parkinson disease (PD). However, little is known about the association between the presence of extra-nigral pathological conditions and freezing of gait (FoG). Patients with PD (n = 143; age, 65.5 ± 7.4 years, Hoehn and Yahr stage, 2.4 ± 0.6; Montreal Cognitive Assessment score, 25.9 ± 2.6) underwent [^{11}C]methyl-4-piperidinyl propionate acetylcholinesterase and [^{11}C]dihydrotetrabenazine dopaminergic PET imaging, and clinical, including FoG, assessment in the dopaminergic "off" state. A subset of subjects (n = 61) underwent [^{11}C]Pittsburgh compound-B β-amyloid positron emission tomography (PET) imaging. Normative data were used to dichotomize abnormal β-amyloid uptake or cholinergic deficits. Freezing of gait was present in 20 patients (14.0%). Freezers had longer duration of disease ($P = 0.009$), more severe motor disease ($P < 0.0001$), and lower striatal dopaminergic activity ($P = 0.013$) compared with non-freezers. Freezing of gait was more common in patients with diminished neocortical cholinergic innervation (23.9%, $\chi^2 = 5.56$, $P = 0.018$), but not in the thalamic cholinergic denervation group (17.4%, $\chi^2 = 0.26$, $P = 0.61$). Subgroup analysis showed higher frequency of FoG with increased neocortical β-amyloid deposition (30.4%, Fisher Exact test: $P = 0.032$). Frequency of FoG was lowest with absence of both pathological conditions (4.8%), intermediate in subjects with single extra-nigral pathological condition (14.3%), and highest with combined neocortical cholinopathy and amyloidopathy (41.7%; Cochran-Armitage trend test, $Z = 2.63$, $P = 0.015$). Within the group of freezers, 90% had at least one of the two extra-nigral pathological conditions studied. Extra-nigral pathological conditions, in particular the combined presence of cortical cholinopathy and amyloidopathy, are common in PD with FoG and may contribute to its pathophysiology.

Key Words: acetylcholine; β-amyloid; dopamine; gait freezing; Parkinson's disease; PET

*Correspondence to: Nicolaas I. Bohnen, MD, PhD, Functional Neuroimaging, Cognitive and Mobility Laboratory, Departments of Radiology and Neurology, University of Michigan, 24 Frank Lloyd Wright Drive, Box 362, Ann Arbor, MI 48105-9755, USA, E-mail: nbohnen@umich.edu

Funding agencies: This study was supported by National Institutes of Health grants P01 NS015655 & R01 NS07085, the Michael J. Fox Foundation, and the Department of Veterans Affairs.

Relevant conflicts of interest/financial disclosures: Nothing to report. Full financial disclosures and author roles may be found in the online version of this article.

Received: 2 February 2014; **Revised:** 28 April 2014; **Accepted:** 4 May 2014

Published online 7 June 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.25929

Advancing Parkinson's disease (PD) is associated with disabling axial motor complications, such as freezing of gait (FoG), with diminishing responsiveness to dopaminergic medications.[1,2] Nigrostriatal dopaminergic denervation secondary to α-synuclein related pathology is the defining pathological feature of most early-stage PD clinical manifestations.[3] Heterogeneous involvement of other projection systems and other pathological conditions variably exacerbate the effects of nigrostriatal denervation and is a significant contributor to the varied clinical features of advancing PD. Unlike the uniform presence of striatal dopaminergic denervation in

clinically manifest PD, for example, significant heterogeneity is seen in the range of cholinergic denervation seen in nondemented PD patients.[4] Neocortical cholinergic denervation is present only in approximately one third of nondemented PD patients when compared with normal control subjects.[4] Comorbid β-amyloid plaques also are variably present in PD.[5]

We previously identified pedunculopontine nucleus (PPN)-thalamic and forebrain cortical cholinergic projection system degeneration as a prominent factor related to falls and gait slowing in PD, respectively.[6,7] We recently found also that cortical β-amyloid deposition is associated with severity of postural instability and gait difficulties features in PD.[8]

We set out to further refine these observations and test the hypothesis that extra-nigral changes of subcortical or neocortical cholinergic denervation (cholinopathy) and increased β-amyloid deposition (amyloidopathy) are factors associating specifically with FoG in PD.

Subjects and Methods

Subjects and Clinical Test Battery

This cross-sectional study involved 143 subjects with PD (106 men and 37 women); mean age, 65.5 ± 7.4 (range, 50-84) years. The PD subjects met the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria.[9] Fifty PD subjects were taking a combination of dopamine agonist and carbidopa-levodopa medications, 67 were using carbidopa-levodopa alone, 15 were taking dopamine agonists alone, and 11 were not receiving dopaminergic drugs. No subjects were treated with anti-cholinergic or cholinesterase inhibitor drugs. Most subjects had moderate severity of disease: 4 patients in stage 1, 7 in stage 1.5, 35 in stage 2, 63 in stage 2.5, 30 in stage 3, 3 in stage 4, and 1 in stage 5 of the modified Hoehn and Yahr classification.[10] Mean duration of disease was 6.0 ± 4.3 (SD; range, 0.5-20) years. The mean motor examination score on the Movement Disorder Society Revised Unified Parkinson's Disease Rating Scale (MDS-UPDRS) was 32.5 ± 14.2 (range, 8-72). Subjects receiving dopaminergic medications were examined in the morning after dopaminergic medications had been withheld overnight. The presence or absence of FoG was based on clinical examination and directly observed by the clinician examiner based on a non-zero score on item 3.11, "Freezing of Gait," of the MDS-UPDRS motor examination.[11] For most reliable assessment, FoG classification should be based on objective confirmation by an experienced observer during clinical assessment rather than on patient recollection.[12] Details of gait speed, UPDRS motor, and cognitive testing from this cohort of patients are reported elsewhere.[4,8,13] All PD subjects previously reported in the quantitative studies by Petrou et al.[13] (n = 40) and Muller et al.[8] (n = 44) are included in the expanded dataset.[8,13]

Each patient underwent neuropsychological examination as described previously.[4] Global cognitive composite *z*-scores were calculated based on normative data. Patients also completed a Montreal Cognitive Assessment, with a mean score of 25.9 ± 2.6 (range, 15-30).[14]

This study was approved by the Institutional Review Board of the University of Michigan School of Medicine. Written informed consent was obtained from all subjects.

Imaging Techniques

All subjects underwent brain magnetic resonance imaging (MRI) and methyl-4-piperidinyl propionate (PMP) acetylcholinesterase and [^{11}C]dihydrotetrabenazine (DTBZ) vesicular monoamine transporter type 2 (VMAT2) positron emission tomography (PET), except for one subject for whom the DTBZ PET scan failed because of technical reasons. All DTBZ PET scans showed evidence of nigrostriatal degeneration. A subset of PD subjects (n = 61) who were clinically identified as being at increased risk for development of dementia based on older age, subjective cognitive complaints, and more severe motor disease also underwent [^{11}C]Pittsburgh compound B (PIB) β-amyloid PET imaging.

[^{11}C]dihydrotetrabenazine PET imaging was performed the morning after withholding dopaminergic medications overnight. The MRI was performed on a 3 Tesla Philips Achieva system (Philips, Best, The Netherlands), and PET imaging was performed in 3D imaging mode with an ECAT Exact HR+ tomograph (Siemens Molecular Imaging, Inc., Knoxville, TN) as previously reported.[4]

The [^{11}C]DTBZ, [^{11}C]PMP, and [^{11}C]PIB were prepared as described previously.[15,16] Dynamic PET scanning was performed for 70 minutes using a bolus dose of 15 mCi [^{11}C]PMP dose. Bolus/infusion protocols were used for [^{11}C]DTBZ (15 mCi) in 60 minutes and for [^{11}C]PIB (18 mCi) in 70 minutes.[17]

Analysis

All image frames were spatially coregistered within subjects with a rigid-body transformation to reduce the effects of subject motion during the imaging session.[18] Interactive Data Language image analysis software (Research Systems, Inc., Boulder, CO) was used to manually trace volumes of interest on MRI images to include the thalamus, caudate nucleus, and putamen of each hemisphere. Total neocortical volumes of interest were defined using semi-automated threshold delineation of the cortical gray matter signal on the MRI scan[4].

[^{11}C]dihydrotetrabenazine distribution volume ratios were estimated using the Logan plot graphical analysis

TABLE 1. Mean (±SD) values of demographic, clinical, cognitive and striatal [^{11}C]dihydrotetrabenazine VMAT2 distribution volume ratios in the patients with PD without versus with FoG[a]

	PD Without FoG (n = 123)	PD With FoG (n = 20)	Statistical Significance
Age	65.4 ± 7.7	66.4 ± 6.0	$t = 0.58$; $p = 0.56$
Sex (females/males)	34/89	3/17	$\chi^2 = 1.43$; $p = 0.23$
Duration of motor disease(y)	5.6 ± 4.1	8.3 ± 4.7	$t = 2.65$; $p = 0.009$
Hoehn and Yahr stage	2.4 ± 0.5	3.0 ± 0.7	$t_{approx} = 5.23$; $p < 0.0001$
Motor MDS-UPDRS	30.3 ± 12.9	46.7 ± 14.0	$t = 5.21$; $p < 0.0001$
Montreal Cognitive Assessment	26.0 ± 2.6	25.3 ± 2.7	$t = 1.18$; $p = 0.24$
Global cognitive composite *z*-score	−0.34 ± 0.87	−0.75 ± 1.06	$t = 1.92$; $p = 0.058$
Striatal VMAT2 binding	1.96 ± 0.27	1.81 ± 0.27	$t = 2.53$, $p = 0.013$

[a]Gender distribution is presented as proportions. Levels of statistical difference between groups are also presented.
Note: t_{approx} = Satterthwaite's method of approximate *t* tests.
Abbreviations: VMAT2, vesicular monoamine transporter type 2; MDS-UPDRS, Movement Disorder Society Revised Unified Parkinson's Disease Rating Scale.

method with the striatal time activity curves as the input function and the total neocortex as reference tissue, a reference region overall low in VMAT2 binding sites, with the assumption that the nondisplaceable distribution is uniform across the brain at equilibrium.[19]

Acetylcholinesterase [^{11}C]PMP hydrolysis rates (k_3) were estimated using the striatal volume of interest (defined by manual tracing on the MRI scan of the putamen and caudate nucleus) as the tissue reference for the integral of the precursor delivery.[20] There are two major brain cholinergic projection systems: the basal forebrain complex, providing the principal cholinergic input to the cortical mantle, and the PPN complex, providing cholinergic inputs to the thalamus, cerebellum, basal ganglia, other brainstem nuclei, and the spinal cord.[21] Both degenerate variably in PD. Acetylcholinesterase PET imaging assesses cholinergic terminal integrity with cortical uptake, reflecting largely basal forebrain neuron integrity, and thalamic uptake, principally reflecting PPN integrity.

[^{11}C]PIB PET data were analyzed using the Logan graphical method and the cerebellum as reference regions.[22,23] Global cortical PIB distribution volume ratio was calculated.

To determine the frequency of each of the two extra-nigral pathological conditions, we used normative data to dichotomize abnormal uptake or deficits.

We classified acetylcholinesterase PET scans as either below or within normal range because of the significant overlap of cholinergic innervation seen between subjects with PD and healthy controls. Abnormal neocortical and thalamic cholinergic innervation was based on a 5th percentile cutoff from non-PD elderly normative data (n = 29; mean age, 66.8 ± 10.9 years; comparable to the current patient cohort).[4]

We used [^{11}C]PIB β-amyloid data from young to middle-aged healthy subjects (n = 115 men/6 women), mean age 46.7 ± 3.4 (range, 40-50 years) to define elevated [^{11}C]PIB binding in the patients, because significant fibrillary β-amyloid deposition occurs in many cognitive normal elderly individuals.[24] The maximum normal neocortical [^{11}C]PIB distribution volume ratio was 1.17, and PD patients with values exceeding this threshold were defined as exhibiting increased β-amyloid binding.

Standard pooled-variance t or Satterthwaite's method of approximate *t* tests (t_{approx}) were used for group comparisons. Chi-squared testing was performed for comparison of proportions between groups and with Fisher's exact test in the presence of small cell populations. The Cochran-Armitage trend test was performed to determine a dose–response relationship when assessing the association between no, single, or dual extranigral pathological conditions and FoG status. In the subset of subjects who had completed all three PET measures, multiple logistic regression was performed, using FoG status as the outcome parameter, and cholinopathy, amyloidopathy, striatal VMAT2 bindings as PET regressors and clinical confounder variables (motor, cognitive performance, and duration of disease) that were shown to be different between freezers and non-freezers. Analyses were performed using SAS version 9.2, SAS Institute, Cary, NC, USA).

Results

Patients With FoG

Twenty subjects had observed FoG (14.0%). Freezers had longer duration of disease, more severe motor impairments, and lower striatal dopaminergic activity compared with non-freezers (Table 1). No significant differences were seen in age or sex distribution between groups. Freezers manifested a trend toward lower cognitive performance compared with non-freezers (Table 1).

Cholinergic PET Findings in Freezers

Neocortical cholinergic denervation was heterogeneous, with 46 PD patients (32.2%) exhibiting neocortical acetylcholinesterase activity below normal-range activity levels. Below-normal-range PPN-thalamic acetylcholinesterase activity levels were seen in 23

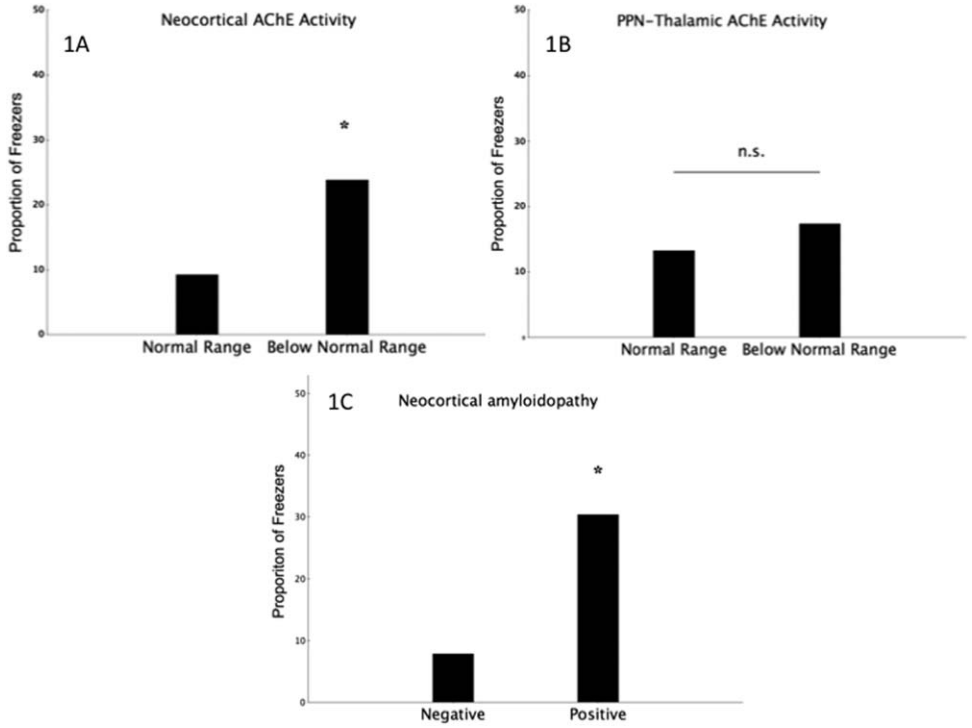

FIG. 1. Proportion of patients (%) with FoG in normal range versus below-range cortical (**A**), and PPN-thalamic cholinergic activity (**B**). FoG was more common in patients with diminished neocortical cholinergic innervation but not in the thalamic cholinergic denervation group. Subgroup analysis of patients who completed β-amyloid PET showed higher frequency of FoG in patients with positive compared with negative neocortical β-amyloid deposition (**C**). Note: *Statistical significant group difference, $P < 0.05$. AChE, acetylcholinesterase; FoG, freezing of gait; PPN, pedunculopontine nucleus; PET, positron emission tomography.

patients (16.1%). A significantly higher proportion of FoG subjects were in the below-normal range compared with the normal-range neocortical acetylcholinesterase group: 23.9% versus 9.3% ($\chi^2 = 5.56$, $P = 0.018$, Fig. 1A). There was no significant difference in the proportion of freezers in the below-normal range versus normal-range PPN-thalamic acetylcholinesterase group: 17.4% versus 13.3% ($\chi^2 = 0.26$, $P = 0.61$; Fig. 1B).

Subgroup Analysis of β-Amyloid PET Findings in Freezers

A subset of PD subjects (n = 61) underwent also [^{11}C]PIB β-amyloid PET imaging. Subjects in this subgroup were older (69.0 ± 6.3 vs 62.9 ± 7.1), had more severe cognitive impairments (composite *z*-score −0.87 ± 0.96 vs −0.04 ± 0.68) and slightly higher Hoehn and Yahr stage (2.7 ± 0.5 vs 2.3 ± 0.6) compared with the remainder of the subjects (n = 82). However, no significant difference was seen in the frequency of FoG between these two subgroups (16.4% vs 12.2% ($\chi^2 = 0.51$, $P = 0.44$). Increased neocortical β-amyloid accumulation was present in 23 of the 61 subjects (37.7%) who completed the [^{11}C]PIB scan. A significantly higher proportion of patients with FoG were in the "positive" compared with the "negative" neocortical β-amyloid group: 30.4% versus 7.9% (Fisher's exact test: $P = 0.032$; Fig. 1C).

Proportion of Freezers in Subgroup Combinations of Patients With Cortical Amyloidopathy or Cholinopathy

No significant association was found between the presence of cortical cholinopathy and amyloidopathy ($\chi^2 = 0.32$, $P = 0.58$); 47.8% of subjects with amyloidopathy have normal-range neocortical cholinergic innervation, and 52.2% have below-normal-range activity. Combined pathological conditions were present in 19.7% of patients, absence of both in 34.4%, with the remainder exhibiting either isolated cholinopathy (27.9%) or amyloidopathy (18%).

FoG frequency was lowest in subjects with absence of both pathological conditions (4.8%), intermediate in subjects with single extra-nigral pathological condition (14.3%), and highest with combined neocortical cholinopathy and amyloidopathy (41.7%; Fisher's exact test: $P = 0.03$). A significant trend was seen indicating a dose–response association between absence and presence of single and dual extra-nigral pathological conditions (Cochran-Armitage trend test $Z = 2.63$, $P = 0.015$; Fig. 2). Within the group of freezers, 90% of all freezers had at least one of the two extra-nigral pathological conditions studied. Figure 3 illustrates low-range cortical acetylcholinesterase and "positive" β-amyloid imaging findings in PD freezer compared with the non-freezer.

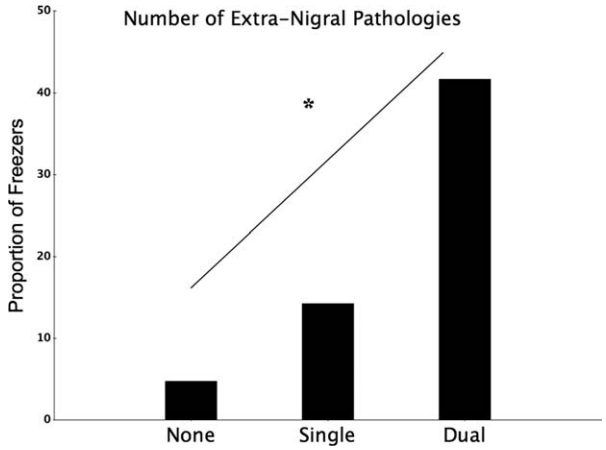

FIG. 2. Proportion of freezers (%) in subgroups of patients with absence, single, or combined presence of cortical amyloidopathy or cholinopathy. A significant trend effect was seen, with FoG frequency being lowest with absence of both pathological condition, intermediate in subjects with single extra-nigral pathological condition, and highest with combined neocortical cholinopathy and amyloidopathy (Cochran-Armitage trend test, $Z = 2.63$; $P = 0.015$). *Statistical significance of trend, $P < 0.05$. FoG, freezing of gait.

Multiple logistic regression analysis was performed to evaluate the relative effects of neocortical amyloidopathy versus cholinopathy while controlling for effects of potentially confounding variables, including striatal VMAT2 binding, estimated duration of motor disease, MDS-UPDRS motor score, and composite cognitive z-score (Table 2). No significant difference in age was found between freezers (69.2 ± 6.7) and non-freezers (68.0 ± 4.1; $t = 0.56$, $P = 0.58$) in this subgroup. An overall significant model ($\chi^2 = 17.66$; $P = 0.0072$) was seen with neocortical amyloidopathy as the only significant predictor variable, whereas neocortical cholinopathy was no longer significant.

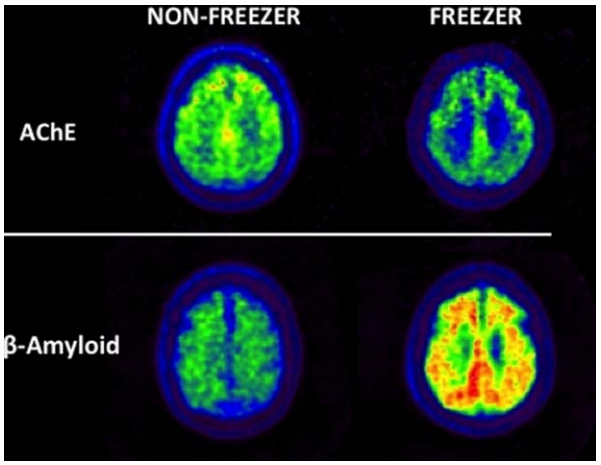

FIG. 3. AChE (upper row) and β-amyloid imaging (lower row) findings in PD non-freezer (left) and freezer (right). Images illustrate reduced cortical cholinergic activity and increased β-amyloid deposition in the freezer compared with the non-freezer. AChE, acetylcholinesterase; PD, Parkinson's disease. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Nigrostriatal dopaminergic denervation had borderline significance in the model ($P = 0.057$).

A post hoc analysis was performed to evaluate the prediction of FoG status based on absolute neocortical β-amyloid [^{11}C]PIB distribution volume ratio rather than as a binary classification variables. Results of the multivariate logistic regression analysis (total model $\chi^2 = 17.26$; $P = 0.0084$), using the absolute neocortical β-amyloid [^{11}C]PIB distribution volume ratio as a predictor variable, confirmed the binary amyloidopathy parameter findings ($\chi^2 = 3.85$; $P = 0.049$).

Discussion

Freezing of gait is a debilitating feature of PD, with poorly defined pathophysiological mechanisms. Sudden freezing may be related to altered neocortical regulation of movement, perhaps exacerbated by progressive impairment of subcortical locomotor centers function.[25] Several studies explored the sources of altered cerebral functions associated with FoG. These studies show evidence of disruption of cortical function, including neocortical networks involved in executive functions and sensorimotor perception in subjects who tend to have severe nigrostriatal denervation, particularly of the caudate nucleus.[25-27] A recent resting state functional MRI brain connectivity study identified reduced cortical network connectivity in PD with FoG, consistent with a role for cortical dysfunction in FoG.[27] Localization of the key nodes within the locomotor network whose dysfunction is responsible for FoG remains unclear. Recent studies emphasize altered interactions between subcortical, in particular the PPN, and cortical regions. For example, one recent functional MRI study of PD subjects with known FoG during a virtual reality timed "up and go" gait task provides evidence of dysfunction across coordinated neural networks, including the caudate nucleus, globus pallidus pars interna, thalamus, and mesencephalic locomotor center.[28] A recent diffusion tensor imaging study showed evidence of reduced connectivity of the PPN and cortical regions.[29] Similarly, a PPN deep brain stimulation study showed significant regional cerebral blood flow increments not only to subcortical but also to cortical regions, including the sensorimotor and supplemental motor cortices.[30]

The main findings in this study reflect an association between presence of observed FoG and extra-nigral pathological conditions. However, mechanistic or etiopathogenetic inferences cannot be drawn from these observations. Furthermore, our PET markers may indicate the presence of a cortical pathological condition but cannot explain the episodic nature of the FoG movement disorder. In this respect, our findings may identify a weak link within a neural circuit, where freezing behavior in PD may occur because of

TABLE 2. Results of multiple logistic regression analysis and FoG in PD patients[a]

Variable	Logit Regression Coefficient	Standard Error	χ^2; p Value
Cortical acetylcholinesterase group status (below versus normal range)	0.31	0.48	0.41; $p = 0.52$
Cortical β-amyloid status(positive versus negative)	0.95	0.48	3.97; $p = 0.046$
Striatal VMAT2 distribution volume ratio	−6.40	3.36	3.63; $p = 0.057$
Duration of motor disease	−0.05	0.09	0.28; $p = 0.59$
Motor MDS-UPDRS score	0.05	0.03	2.23; $p = 0.14$
Global cognitive composite *z*-score	0.48	0.55	0.76; $p = 0.38$

[a]Overall model was significant ($\chi^2 = 17.66$; $P = 0.0072$).
Abbreviations: VMAT2, vesicular monoamine transporter type 2; MDS-UPDRS, Movement Disorder Society Revised Unified Parkinson's Disease Rating Scale.

impaired communication between complementary yet competing neural networks.[31,32]

Our findings support the role of neocortical changes in PD patients with FoG. We found that the effects of cholinergic projection system deficits associated with FoG were driven by neocortical denervation but not by PPN-thalamic degeneration. Degeneration of cholinergic PPN-thalamic projections is associated with postural reflex impairments, whose underlying pathophysiology may differ from that leading to FoG.[33,34] However, PPN-thalamic and forebrain cortical cholinergic denervation do partially overlap,[4] and a potential role of PPN-thalamic cholinergic denervation cannot be excluded.

We recently reported a relationship between postural instability and gait difficulty feature severity and neocortical β-amyloid burden in PD patients at risk for development of dementia.[8] Similar findings have been reported for lower cerebrospinal fluid β-amyloid concentrations in patients with PD and the postural instability and gait difficulty motor phenotype.[35] These associations of cerebrospinal fluid β-amyloid concentrations with severity of postural instability, gait difficulty, and lower limb bradykinesia were also independent from age and cognitive functions in PD patients, and no association between cerebrospinal fluid markers and other motor features were found.[35]

Findings of lack of significant cholinergic status predictor of FoG in the multivariate model limited to the subgroup of 61 patients who completed all three PET scans should be considered as preliminary, given the smaller sample size and because concurrent presence of amyloidopathy and cholinopathy showed a significant dose–response effect in a trend analysis comparing FoG status relative to absence or presence of one or two of these extra-nigral pathological conditions The preliminary multivariate logistic regression analysis in the subset of 61 subjects who completed all three PET scans identified amyloidopathy as a predictor of FoG independent of the degree of cognitive impairment and other clinical confounder variables. Although the extra-nigral pathological conditions of cortical cholinopathy and amyloidopathy are common in subjects with FoG, they are not exclusively present in FoG: other pathological conditions or mechanisms also may play a role. These preliminary observations need to be confirmed in future prospective studies with semiquantitative assessment of FoG, using dedicated provocation protocols,[12] preferably performed in both the dopaminergic medication "on" and "off" states.

We found that freezers had more severe nigrostriatal denervation compared with non-freezers in our study, and nigrostriatal denervation had borderline significant prediction of FoG in the multifactorial model. This is in keeping with the fact that FoG can respond to dopaminergic medications in some subjects,[36] such as in patients with less severe disease. These findings emphasize multi-system changes underlying FoG in PD, which is supported by our findings of a dose–response relationship between the higher frequency of FoG in subjects with combined extra-nigral pathological conditions, compared with a single presence or absence of both. Relatively isolated impairment of a single system in PD (i.e., nigrostriatal denervation) may not lead to the development of some motor impairments because of adaptive plasticity in the remaining intact systems. The degree of dopamine responsiveness of gait freezing may depend on the integrity and adaptive plasticity of the remainder of the neural networks supporting mobility functions.

A limitation of our study is that assessment of FoG was based on an unprovoked protocol with no special maneuvers such as walking through narrow passages or making turns. This may limit the sensitivity of our FoG assessment and may explain the relatively low frequency of FoG in our study population. This low sensitivity also may result in a relative underestimation of effect size estimates. A relative advantage is the high specificity of FoG classification. Patients on cholinesterase inhibitors were not eligible for this study because we were performing acetylcholinesterase PET imaging. This exclusion criterion resulted in a predominantly nondemented cohort and may provide an alternative explanation for the relatively low frequency of FoG in our study. Another limitation of the study is that the subset of 61 PD subjects who underwent all three PET scans were older, had greater cognitive impairments, and had slightly higher Hoehn and Yahr

stages compared with the remainder of the subjects. However, no significant difference in the frequency of FoG was found between these two subgroups. Furthermore, despite the fact that all subjects in this subgroup had more significant cognitive impairment, the frequency of amyloidopathy in subjects with FoG was nearly fourfold higher compared with the non-FoG at-risk subjects. We conclude that cortical cholinopathy and amyloidopathy are common extra-nigral pathological conditions in PD with FoG and in the presence of more severe nigrostriatal degeneration may contribute to its pathophysiology. ■

Acknowledgements: We thank all patients for their time commitment. We also thank Christine Minderovic, Cyrus Sarosh, Virginia Rogers, the PET technologists, cyclotron operators, and chemists, for their assistance.

References

1. Lopez IC, Ruiz PJ, Del Pozo SV, Bernardos VS. Motor complications in Parkinson's disease: ten year follow-up study. Mov Disord 2010;25:2735-2739.
2. Vu TC, Nutt JG, Holford NH. Progression of motor and nonmotor features of Parkinson's disease and their response to treatment. Br J Clin Pharmacol 2012;74:267-283.
3. Frey KA, Koeppe RA, Kilbourn MR, et al. Presynaptic monoaminergic vesicles in Parkinson's disease and normal aging. Ann Neurol 1996;40:873-884.
4. Bohnen NI, Muller MLTM, Kotagal V, et al. Heterogeneity of cholinergic denervation in Parkinson disease J Cereb Blood Flow Metab 2012;32:1609-1617.
5. Donaghy P, Thomas AJ, O'Brien JT. Amyloid PET imaging in Lewy body disorders. Am J Geriatr Psychiatry 2013. DOI: 10.1016/j.jagp.2013.03.001.
6. Bohnen NI, Muller ML, Koeppe RA, et al. History of falls in Parkinson disease is associated with reduced cholinergic activity. Neurology 2009;73:1670-1676.
7. Bohnen NI, Frey KA, Studenski S, et al. Gait speed in Parkinson disease correlates with cholinergic degeneration. Neurology 2013; 81:1611-1616.
8. Muller ML, Frey KA, Petrou M, et al. β-amyloid and postural instability and gait difficulty in Parkinson's disease at risk for dementia. Mov Disord 2013;28:296-301.
9. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinicopathologic study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181-184.
10. Goetz CG, Poewe W, Rascol O, et al. Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. Mov Disord 2004;19:1020-1028.
11. Goetz CG, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): process, format, and clinimetric testing plan. Mov Disord 2007;22:41-47.
12. Snijders AH, Haaxma CA, Hagen YJ, Munneke M, Bloem BR. Freezer or non-freezer: clinical assessment of freezing of gait. Parkinsonism Relat Disord 2012;18:149-154.
13. Petrou M, Bohnen NI, Muller ML, Koeppe RA, Albin RL, Frey KA. Abeta-amyloid deposition in patients with Parkinson disease at risk for development of dementia. Neurology 2012;79:1161-1167.
14. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53:695-699.
15. Shao X, Hoareau R, Hockley BG, et al. Highlighting the Versatility of the Tracerlab Synthesis Modules. Part 1: Fully automated production of [F]labelled radiopharmaceuticals using a Tracerlab FX(FN). J Labelled Comp Radiopharm 2011;54:292-307.
16. Klunk WE, Engler E, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease using the novel positron emission tomography tracer, Pittsburgh Compound-B. Ann Neurol 2004;55:306-319.
17. Koeppe RA, Frey KA, Kume A, Albin R, Kilbourn MR, Kuhl DE. Equilibrium versus compartmental analysis for assessment of the vesicular monoamine transporter using (+)-alpha-[^{11}C]dihydrotetrabenazine (DTBZ) and positron emission tomography. J Cereb Blood Flow Metab 1997;17:919-931.
18. Minoshima S, Koeppe RA, Fessler JA, et al. Integrated and automated data analysis method for neuronal activation studying using O^{15} water PET. In: Uemura K, Lassen NA, Jones T, Kanno I, eds. *Quantification of Brain Function to Tracer Kinetics and Image Analysis in Brain PET*. Tokyo: Excerpta Medica, 1993: 409-418.
19. Koeppe RA, Frey KA, Kuhl DE, Kilbourn MR. Assessment of extrastriatal vesicular monoamine transporter binding site density using stereoisomers of [^{11}C]dihydrotetrabenazine. J Cereb Blood Flow Metab 1999;19:1376-1384.
20. Nagatsuka S, Fukushi K, Shinotoh H, et al. Kinetic analysis of [(^{11})C]MP4A using a high-radioactivity brain region that represents an integrated input function for measurement of cerebral acetylcholinesterase activity without arterial blood sampling. J Cereb Blood Flow Metab 2001;21:1354-1366.
21. Heckers S, Geula C, Mesulam M. Cholinergic innervation of the human thalamus: dual origin and differential nuclear distribution. J Comp Neurol 1992;325:68-82.
22. Logan J, Fowler JS, Volkow ND, Ding YS, Wang GJ, Alexoff DL. A strategy for removing the bias in the graphical analysis method. J Cereb Blood Flow Metab 2001;21:307-320.
23. Price JC, Klunk WE, Lopresti BJ, et al. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh compound-B. J Cereb Blood Flow Metab 2005;25:1528-1547.
24. Aizenstein HJ, Nebes RD, Saxton JA, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. Arch Neurol 2008;65:1509-1517.
25. Snijders AH, Leunissen I, Bakker M, et al. Gait-related cerebral alterations in patients with Parkinson's disease with freezing of gait. Brain 2011;134:59-72.
26. Bartels AL, Leenders KL. Brain imaging in patients with freezing of gait. Mov Disord 2008;23 Suppl 2:S461-467.
27. Tessitore A, Amboni M, Esposito F, et al. Resting-state brain connectivity in patients with Parkinson's disease and freezing of gait. Parkinsonism Relat Disord 2012;18:781-787.
28. Shine JM, Matar E, Ward PB, et al. Exploring the cortical and subcortical functional magnetic resonance imaging changes associated with freezing in Parkinson's disease. Brain 2013;136:1204-1215.
29. Fling BW, Cohen RG, Mancini M, Nutt JG, Fair DA, Horak FB. Asymmetric pedunculopontine network connectivity in parkinsonian patients with freezing of gait. Brain 2013;136:2405-2418.
30. Ballanger B, Lozano AM, Moro E, et al. Cerebral blood flow changes induced by pedunculopontine nucleus stimulation in patients with advanced Parkinson's disease: a [(15)O] H2O PET study. Hum Brain Mapp 2009;30:3901-3909.
31. Shine JM, Matar E, Ward PB, et al. Freezing of gait in Parkinson's disease is associated with functional decoupling between the cognitive control network and the basal ganglia. Brain 2013;136:3671-3681.
32. Giladi N, Horak FB, Hausdorff JM. Classification of gait disturbances: distinguishing between continuous and episodic changes. Mov Disord 2013;28:1469-1473.
33. Karachi C, Grabli D, Bernard FA, et al. Cholinergic mesencephalic neurons are involved in gait and postural disorders in Parkinson disease. J Clin Invest 2010;120:2745-2754.
34. Muller ML, Albin RL, Kotagal V, et al. Thalamic cholinergic innervation and postural sensory integration function in Parkinson's disease. Brain 2013;136:3282-3289.
35. Alves G, Larsen JP, Emre M, Wentzel-Larsen T, Aarsland D. Changes in motor subtype and risk for incident dementia in Parkinson's disease. Mov Disord 2006;21:1123-1130.
36. Schaafsma JD, Giladi N, Balash Y, Bartels AL, Gurevich T, Hausdorff JM. Gait dynamics in Parkinson's disease: relationship to Parkinsonian features, falls and response to levodopa. J Neurol Sci 2003;212:47-53.